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**FINAL REPORT**  
**PHOTOSYNTHETIC AND RESPIRATORY ACTIVITY**  
**IN**  
**GERMFREE HIGHER PLANT SPECIES**

**Prepared Under**  
**Contract no. NAS9-12912**

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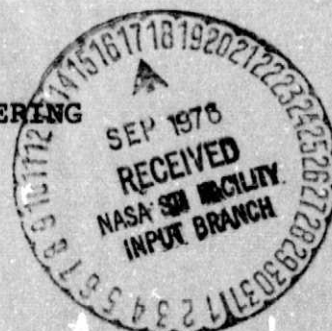
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By  
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RICE UNIVERSITY  
HOUSTON, TEXAS

FOR

JOHNSON SPACE CENTER

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

HOUSTON, TEXAS 77058

JULY 1976

PHOTOSYNTHETIC AND RESPIRATORY ACTIVITY

IN

GERMFREE HIGHER PLANT SPECIES

Contract No. NAS9-12912

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This program, as originally conceived, was to be a cooperative research effort between Rice University and the Johnson Space Center (then known as the Manned Spacecraft Center) to extend the scope of NASA investigations on germfree higher plants. Rice University was to develop equipment and techniques to quantitatively measure gas exchange of germfree plants. Detailed studies of photosynthesis and respiration were to be performed by Rice on germfree plants supplied on a regular basis by NASA from their inhouse germfree plant growth facility. However, due to no fault of the contractor, NASA was unable to supply the germfree plants required for the study.

Since germfree plants were never made available to Rice University, the scope of the study was of necessity limited to development of equipment and techniques. The unique facilities developed as a result of this contractual effort for the study of gas exchange of germfree plants in both stationary and rotational mode are the subject of this Final Report.

## DESIGN OF SYSTEM

### Clinostat

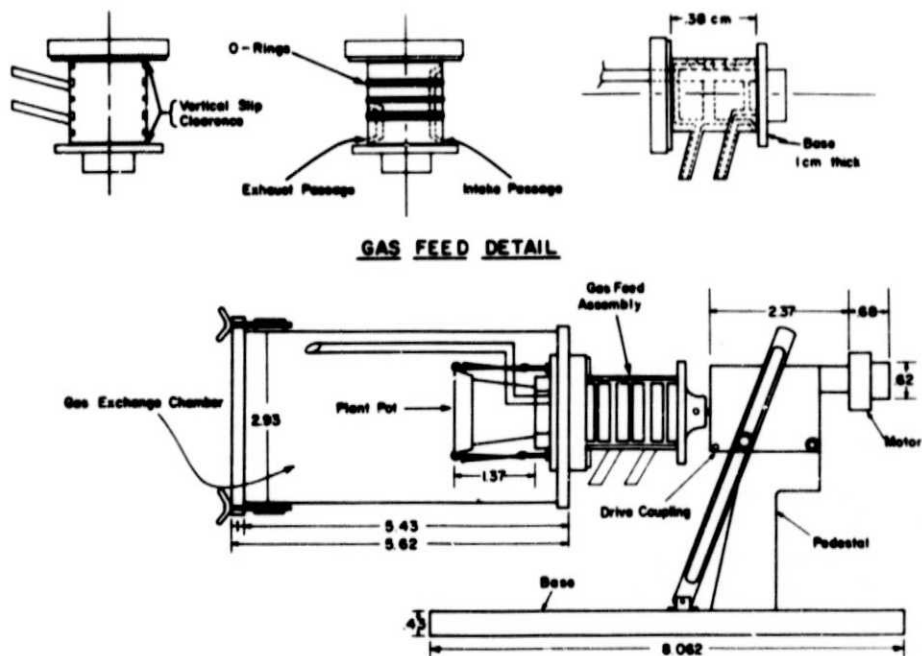
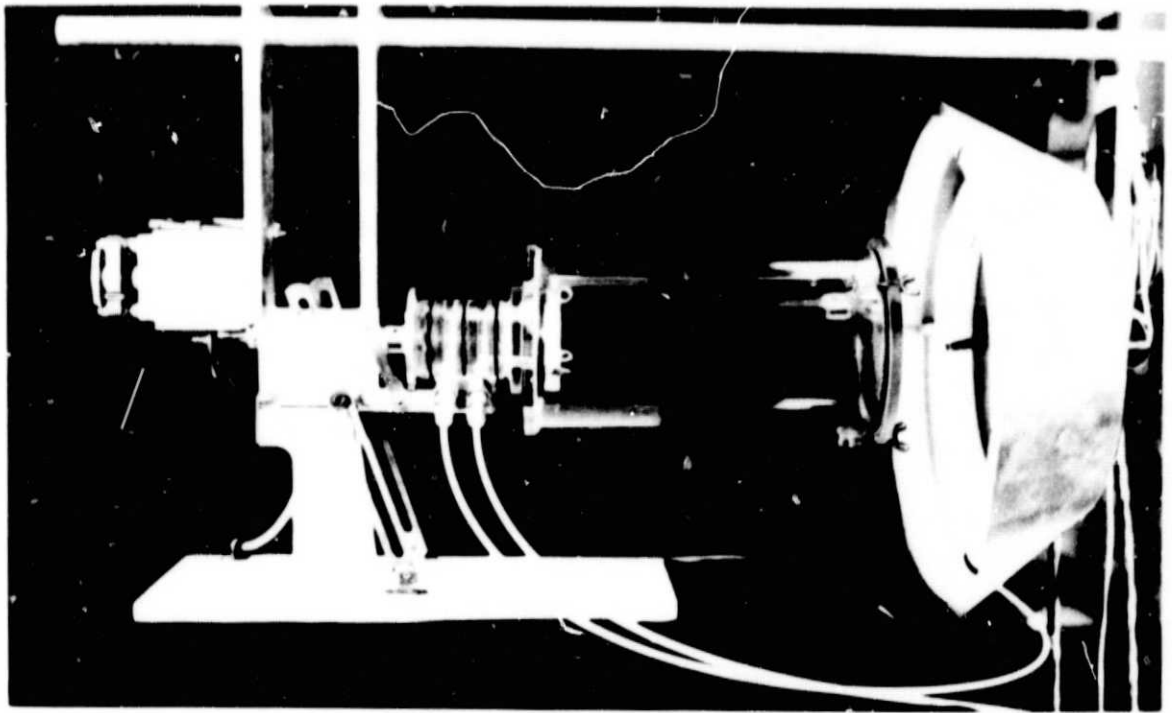
The clinostat, to provide for rotation, consists of an aluminum block, drilled through and fitted with ballbearings in each orifice. A 0.25 rpm Model 425 Bristol Motor (Minarik Electric Co., Los Angeles, California) was mounted on one side of the block. The drive shaft, supported by ballbearings, extends through the block. An aluminum connector is mounted on the free end of the drive shaft to serve as anchorage for the gas feed assembly and gas exchange chamber. The clinostat is hinge mounted to a wooden pedestal which is in turn fastened to a wooden base. The drive assembly and base are connected by a brass tilt lock to permit selection of any clinostat angle between horizontal and vertical. Speed of rotation is varied by use of motors of various size. A schematic drawing and photograph of the clinostat are shown in Figure 1.

### Gas Exchange Chamber

The main body of the gas exchange chamber is made of plexiglass pipe (I.D., 13.6 cm; O.D., 14.3 cm) with a 1 cm thick plexiglass disc cemented to one end to form an air tight base plate (Fig. 1). A smaller plexiglass disc (1.5 cm thick; 90 cm in diameter) is bolted in the geometric center of the chamber base plate. Two holes drilled through the center of the plexiglass disc and chamber base plate serve to attach the core of the gas feed assembly. Holes in the disc and base plate are aligned

FIGURE 1.

SCHEMATIC DRAWING AND PHOTOGRAPH OF  
THE CLINOSTAT AND GAS EXCHANGE CHAMBER.



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to permit gas flow between the gas feed assembly and gas exchange chamber (Fig. 1). Screws attach the gas feed assembly (and chamber) to the aluminum connector of the clinostat drive shaft.

A removable cap was constructed from plexiglass to seal the gas exchange chamber. The cap, concentrically grooved near the outer edge to accommodate an o-ring, forms a gas tight seal with the chamber wall when secured by three wing bolts. Gas tight seals are facilitated by application of silicone grease to the o-ring.

To insure proper mixing of gases within the chamber, the inflow gas orifice is fitted with a plexiglass tube which extends to within 3 cm of the chamber cap. This arrangement forces the inflow gas over the experimental plant before release through the outflow orifice.

#### Gas Feed Assembly

The gas feed assembly is the most important design feature of the gas exchange apparatus. This structure has to permit chamber rotation and simultaneous feed and removal of gas from the chamber without leakage or excessive pressure drop across the system. Several prototypes were evaluated before the assembly described below was adapted for use.

The piston or core of the two-directional gas feed assembly is drilled as indicated in Figure 1 for inlet and outlet gas passages. The piston is equipped with an outer plexiglass sleeve with inlet and outlet ports corresponding positionally with their counterparts in the piston. As indicated in Figure 1, the piston is fitted with o-rings which separate inlet and



outlet gas streams when the sleeve encloses the piston. During rotation the silicon lubricated o-rings maintain gas stream separation while the inlet and outlet ports remain stationary for connection to a gas analytic system.

#### Light Source

During experiments, it is necessary to provide constant illumination perpendicular to the long axis of the experimental plant. To achieve this objective, an adjustable light source was constructed and attached to the gas exchange apparatus (Fig. 1).

The light source consists of a small 15 watt incandescent bulb surrounded by 3 circular cool white fluorescent lamps of differing diameters (20.3 cm, 30.5 cm, and 40.6 cm) mounted in conical formation within an aluminum reflector dome. The entire lamp assembly is mounted above the clinostat chamber by steel bar supports and can be adjusted to required light intensity by raising or lowering the light source. The apparatus is designed to maintain the light source in the same position with respect to the clinostat chamber, whether the chamber is in vertical or horizontal orientation.

#### Enclosure of Plant Pot

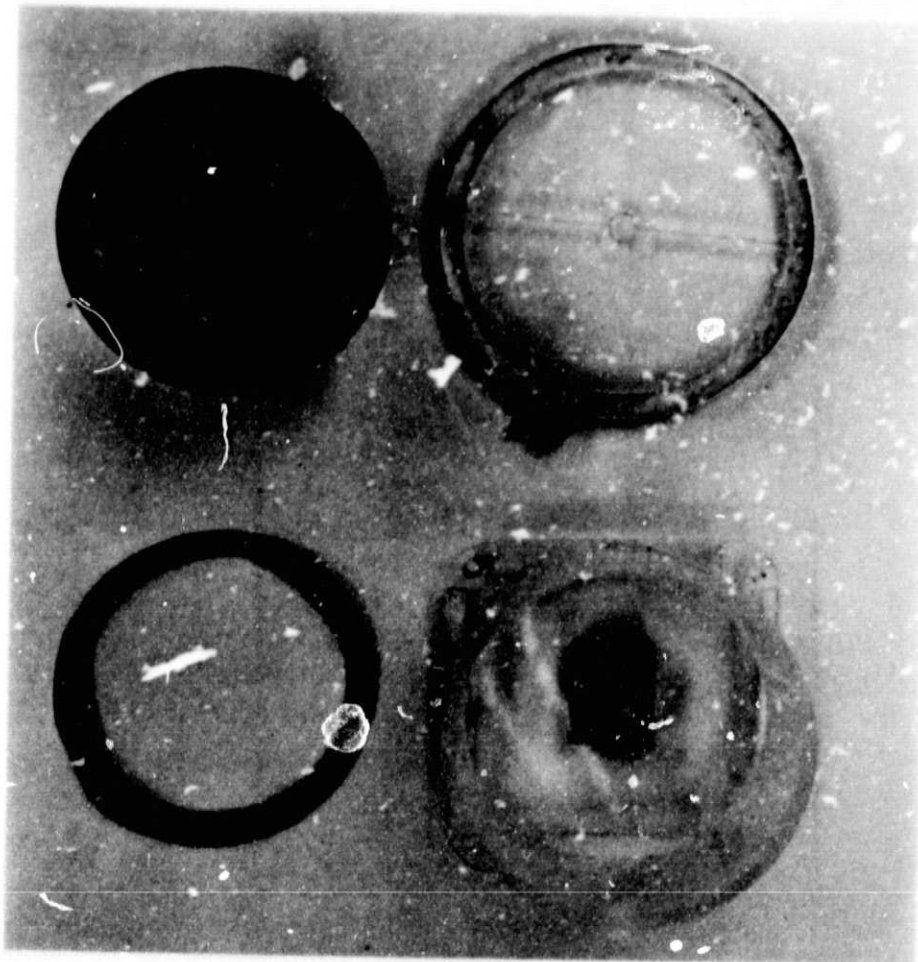
To prevent gas exchange between microorganisms in the potting soil of control plants (not germfree) and the gas chamber atmosphere, a pliable plastic collar is placed around each plant to cover the soil surface. The collar is sealed to the pot and the plant stem with a pliable sealing compound, Apiezon Q (James

G. Biddle Co., Plymouth Meeting, Pa.). The three small holes in the base of each pot are also sealed using this compound. Three fasteners, spring loaded by rubber bands, attached to the chamber base plate hold plastic plant pots in position during experiments. This system works well, but some difficulty is experienced in securing the plant pots inside the chamber. Since the chamber is top loaded, attachment of the fasteners to the plant pot is a cumbersome and often tedious procedure. Hence, another method for sealing and attaching plant pots has been designed.

In this method, the plant pot is placed in a plexiglass cylinder (height, 7.6 cm; I.D., 7.6 cm) which has one end sealed with a plexiglass base plate. An o-ring is positioned on the lip of the cylinder and a plexiglass cap is placed over the cylinder opening, thus enclosing the pot within the cylinder. A slit is made in the cap to accomodate the plant stem. The cap has a lip which overlapps the outer edge of the cylinder and an adjustable metal band is used to tighten the cap over the cylinder. Silicone grease is used to lubricate the o-ring and Apiezon Q is used to seal the silt in the cap. The base plate is drilled in its center for attachment to a screw bolt projecting from the chamber base. The only advantage of this method is ease of securing plant pots inside the chamber. However, this point is important, since undue handling of plants increases the possibility of injury to the plant and subsequent error in experiments. This apparatus is illustrated in Figures 2 and 3. Also, the attachment of this apparatus to the gas chamber is shown in Figure 3.

FIGURE 2.

ISOLATION CHAMBER FOR PLANT POT.



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FIGURE 3.

GAS EXCHANGE SYSTEM.



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### Gas Analytic System

Cylinders of required experimental gases are connected to separate inflow ports of a multiple channel valve by means of flexible nylon tubes (I.D., 0.5 cm). Each line is equipped with a pressure regulator to eliminate excessive pressure surges and to maintain equal pressures in the system. The outflow port of the multiple channel valve is connected to the inflow port of the gas exchange chamber by a short segment of nylon tube. The line to the gas exchange chamber is equipped with a by-pass system so that sample gas can be directed to the CO<sub>2</sub> analyzer (Beckman Model 865 Infrared Analyzer), thus reducing the time required for initial calibration. The outflow line from the gas exchange chamber and the chamber by-pass system are directed through an indication Drierite column to removed moisture produced by plant transpiration. A flow meter is installed in the gas line prior to entry into the analyzer. The reference gas line for the analyzer is also equipped with a pressure regulator and flow meter to insure equal pressure and flow rates in both cells of the analyzer. A "bleed" valve is placed in the chamber outflow line, between the Drierite column and flow meter, to facilitate rapid flushing of the gas exchange chamber. The gas analytic system is illustrated in Figure 4.

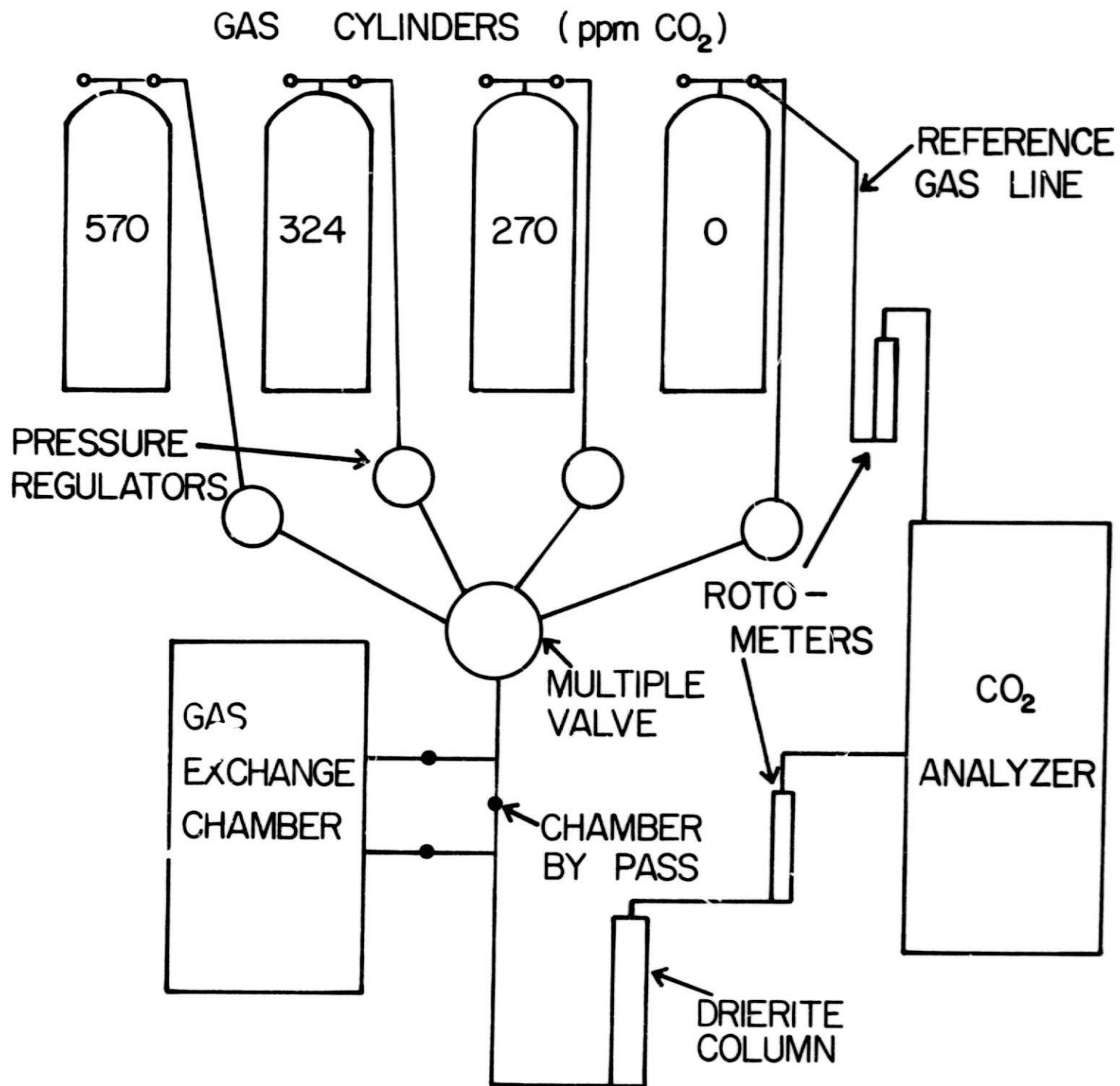
### Temperature Control

The gas exchange chamber and light source are designed for use in a large environmental chamber, while the gas analytic system remains on the outside. Thus, temperature in the gas exchange chamber can be regulated to the desired level.

FIGURE 4.

SCHEMATIC OF GAS ANALYTIC SYSTEM.





## SYSTEM OPERATION

### Evaluation

The gas exchange chamber, the gas analytic system, and the total gas exchange system have been pressurized to 5 psig (3 psig over normal operating pressure). Pressure monitoring with a mercury manometer revealed no leakage after 48 hr. Pressure gauges, installed below the gas exchange chamber showed that the gas feed assembly did not cause a pressure drop in the system. Hence the pressure regulators, valves, and flow meters allow adequate control of gas pressure and flow in the system.

The chamber for isolating the plant pot soil from the gas exchange chamber atmosphere has also been tested. On three occasions, the stem of a plant was excised at soil level, the pot was sealed and placed in the gas exchange chamber. The chamber was flooded with 324 ppm CO<sub>2</sub> in air and the outflow was analyzed with the precalibrated CO<sub>2</sub> analyzer. No changes occurred in the gas concentration after a 3 hr test period, thus indicating no detectable leakage from the plant pot isolation chamber.

### Operation

The CO<sub>2</sub> analyzer is calibrated for the desired range of sensitivity (0 to 600 ppm CO<sub>2</sub> or 250 to 570 ppm CO<sub>2</sub>). The pot of the experimental plant is sealed and secured in the gas exchange chamber. The photocell of a light meter is inserted inside the chamber at the level of the upper leaf surface and the chamber cap is placed loosely in position. The light intensity is then adjusted to the desired level by moving the

adjustable light source. The photocell is removed and the chamber cap sealed in position. Photosynthesis may be measured in both the vertical and horizontal rotating and stationary positions (or at any intermediate angle) until steady state is achieved. Steady state conditions of respiration are determined by turning the light source off and covering the gas exchange chamber with a light-tight black bag. Pressure is maintained at 2 psig and gas flow is set at 140 cc/min.

#### Maintenance

Maintenance of the gas exchange system is insignificant in terms of time involvement and equipment replacement. The only structural items which require replacement are the o-rings in the gas feed assembly and the fluorescent tubes in the light source. However, the gas feed assembly requires frequent lubrication to insure proper functioning and the Drierite must be changed periodically to provide adequate moisture removal.

#### SYSTEM LIMITATIONS

Pressure and flow rates in the analytic system are limited primarily by the operational capacity of the gas analyzer. The gas exchange chamber and gas feed assembly were designed to function at 2 psig but may be used at test pressures up to 5 psig and possibly more. Thus modifications in gas analyzers and structural components in the system could allow for systems operation at pressures and flow rates above those currently permissible. The limited size of the gas exchange chamber also places certain constraints on the experimental plants which may

be used. However, a larger chamber can be used, provided the increased weight does not cause binding in the gas feed assembly.

The gas exchange chamber will not withstand autoclave type sterilization procedures; however, the entire integrated system or individual components (e.g. detached gas exchange chamber only) can be effectively sterilized with ethylene oxide. Loading of germfree plants "behind the barrier" for subsequent "outside" gas exchange experiments is technically feasible with the equipment and techniques developed in this research program.